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TECHNICAL MANUSCRIPT 359

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IN THE RABBIT APPENDIX  
FOLLOWING THYMIC TRANSPLANTS

Alfred C. Standen

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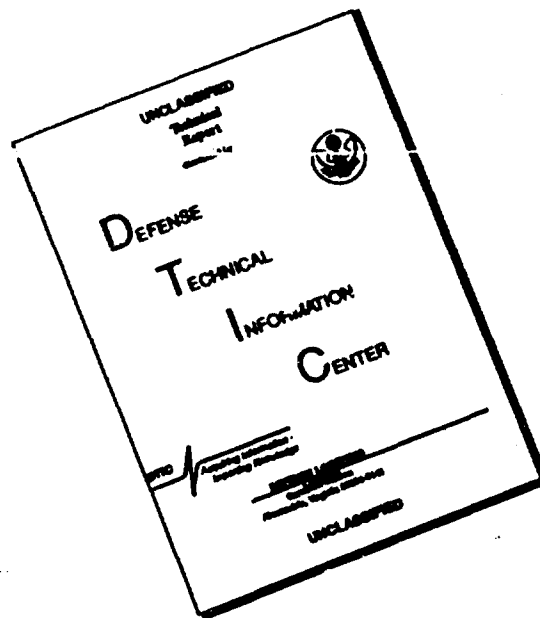
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DEPARTMENT OF THE ARMY  
Fort Detrick  
Frederick, Maryland 21701

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RETICULAR CHANGES IN THE RABBIT  
APPENDIX FOLLOWING THYMIC TRANSPLANTS

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Project 1L013001A91A

February 1967

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

## RETICULAR CHANGES IN THE RABBIT APPENDIX FOLLOWING THYMIC TRANSPLANTS

ABSTRACT

The most striking morphologic similarity between the lymphoid structure of the appendix and the thymus in adult New Zealand rabbits is the constant occurrence of large reticular cell derivatives in the cortico-medullary junction. This can be appreciated in both H&E and PAS preparations. The normal rabbit appendix is an ovoid lymphoid structure covered luminally with one or more layers of modified intestinal epithelium that lacks PAS reactivity but contains foci of lymphocytes. Laterally, the lymphoid structure is separated by thin connective tissue septa carrying blood vessels and lymphatics. When fresh, homologous thymic tissue (1 by 1 cm) was transplanted beneath the kidney capsule, the lymphoid structure showed progressive changes with time between 4 hours and 7 days. The intensity of the reaction was graded, and "t" tests were significant at 0.001. All changes reverted to near normal by 7 days. The PAS reactivity diminished in intensity but not in area with time. No changes occurred in recipient thymus.

The rabbit appendix is a modified lymphoid structure that is morphologically similar in many respects to the thymus. The most striking of these similarities are the large cells of the cortico-medullary junction and the close association of the lymphatic tissue with the overlying cap of epithelium. The similarity between the developing avian bursa of Fabricius and that of the developing lymphoid structure of the rabbit appendix has been noted by Archer and her co-workers in Good's laboratory.\* These investigators have also pointed out functional similarities relative to humoral responses to the injection of bovine gamma globulin when this organ was surgically removed either singly or in combination with other lymphoid tissue.

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\* Archer, O.K.; Pierce, J.C.; Papermaster, B.W.; Good, R.A. 1962. Reduced antibody response in thymectomized rabbits. *Nature* 195:191.

The appendix of the adult rabbit is composed of closely packed ovoid lymphoid structures between which are connective tissue septa containing blood vessels and lymphatics. The luminal surface is capped by modified intestinal epithelium composed of one to several layers of cuboidal cells without nuclear polarization. No goblet cells are present. Lymphocytes within its interstices occur singly, in aggregates, or in rosette formation. A sharp morphological distinction is seen in the region of the crypts of Lieberkühn where this modified epithelium joins the luminal epithelium.

Figure 1 shows a low-power view of the relationship described above. Here one can readily see the close association of the epithelial cap with the lateral borders of the lymphatic structure of the appendix. The difference in the cap epithelium and the mucosal villous epithelium can be seen. The large reticular cells of the cortico-medullary junction are apparent, particularly in areas more distant from the serosal surface. The latter structural relationship is unlike that in other lymphoid tissue with the exception of the thymus.

The cortico-medullary cells can be more easily visualized by their intense PAS reactivity (Fig. 2). The cap epithelium is negative, in contrast to the mucous villous epithelium, which contains strongly positive goblet cells.

The thymus in the adult rabbit contains a similar accumulation of large reticular PAS-reactive cells in the cortico-medullary junction (Fig. 3). These are the same cells that occur in the appendicial lymphoid structure. This relationship can be readily appreciated with both the H&E and PAS staining. This morphological similarity between the appendix and the thymus, which is unlike that in the spleen, mesenteric, and peripheral lymph nodes, was especially pertinent to certain striking reactions noted in subsequent studies. It is these that we wish to report.

New Zealand rabbits were used for this experiment. The appendices of 28 animals were used as controls for semi-quantitative data. The experimental group consisted of rabbits that were anesthetized intravenously with pentobarbital sodium followed by implantation under the kidney capsule of a freshly excised portion of thymus (1 by 1 cm) from a New Zealand rabbit. Each single recipient rabbit received an homologous thymic transplant from only one individual donor rabbit. The experimental recipient rabbits were sacrificed at 4, 24, 48, 72, 96 hours, and 7 days. Sections of the appendices were stained by H&E, PAS, and Giemsa. These appendices were graded for the number of cortico-medullary reticular cells. There was a direct relationship between an increase in these cells and the time of sacrifice, with a peak at 96 hours. At 7 days this reaction was approaching the limits of normal variation. Table 1 shows that, in addition to the progressive increase in cells, the condition becomes markedly intense about the 48th hour, by which time the aggregated cells are confluent. This appearance is even more marked at 96 hours.





Figure 1. Wall of the Appendix, H&E Stain.



Figure 2. PAS Reaction in Wall of the Appendix.

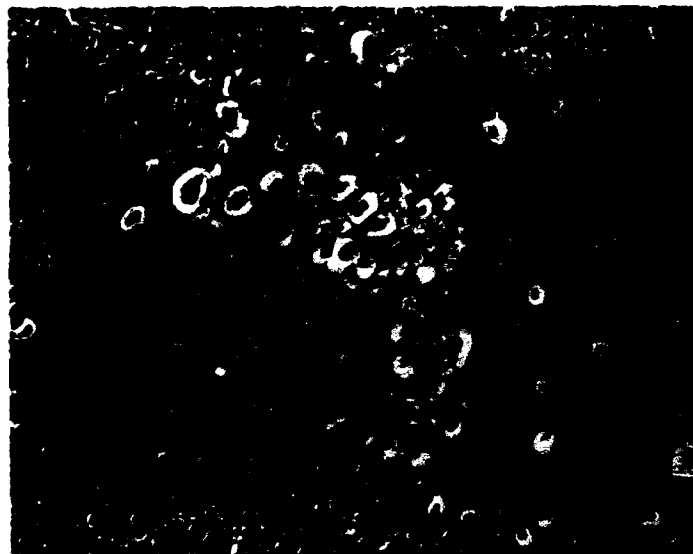


Figure 3. PAS Reaction in Thymus.

TABLE 1. INTENSITY OF CORTICO-MEDULLARY RETICULAR CELLS FOLLOWING HOMOLOGOUS THYMIC TRANSPLANTS

Time	Reticular Cells	Confluence <sup>a</sup> /
Controls	+	-
4 hours	++	-
24 hours	++	-
48 hours	+++	+
72 hours	++++	+++
96 hours	+++++	+++++
7 days	+	-

a. Confluence by the formation of reticular cell aggregates.

The increase in these cells was most prominent in the apical portion, distant from the serosal surface. When the reticular cells in this portion were counted and compared with those of a standard area ( $0.0784 \text{ mm}^2$ ), statistical analysis showed significant differences that could be expressed in terms of student "t" tests. Table 2 shows this relationship.

TABLE 2. RETICULAR CELLS PER  $0.0784 \text{ mm}^2$  AREA

Time	Reticular Cells	p
Controls	$15.4 \pm 0.50$	
4 hours	$21.1 \pm 0.42$	0.001
24 hours	$25.1 \pm 0.60$	0.001
48 hours	$32.2 \pm 1.00$	0.001
72 hours	$27.1 \pm 4.60$	0.001
96 hours	$64.6 \pm 6.10$	0.001
7 days	$20.6 \pm 3.60$	0.01

This transplant reaction can best be appreciated by comparing the control with the 96-hour specimen. Figure 4 shows the control and the 96-hour composite with the H&E stain. At 96 hours there are extensive confluent areas of reticular cell accumulation. Qualitatively the cells are smaller at 96 hours but show more evidence of viability. The nuclear structure is similar. These cells can be traced to reactive proliferation of certain cells of the epithelial cap.

Figure 5 shows the control and a 96-hour specimen with the PAS reaction. It should be noted that the PAS reaction is weaker at 96 hours than in the control, as the number of cells increases. There are no reactions in the thymuses of the recipient animals.



Figure 4. Control (left) and 96-Hour Composite, H&E Stain.



Figure 5. Control (left) and 96-Hour Composite, PAS Stain.

In conclusion, there is a marked reaction involving the cortico-medullary reticular cells of the appendix following the subcapsular transplantation of homologous thymus in the rabbit. This is manifested by a marked increase in cortico-medullary cells and formation of large confluent areas. The cells, which are strongly positive with PAS in the control animals, gradually diminish in reactivity with the duration of transplantation. These cells show more evidence of viability and are smaller. The reaction reaches a maximum at 96 hours and regresses to near normal limits by 7 days. The thymus, which has similar cells with a similar structure relationship, does not give this reaction. This is consistent with the lack of antigen reactivity in the thymus, thought to be due to blood barrier.

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